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TO: Assistant Commissioner for Patents
Box Patent Applications
Washington D.C. 20231

Attorney Docket No. 026083/0195

(must include alphanumeric codes if no inventors named)

UTILITY PATENT APPLICATION TRANSMITTAL
(new nonprovisional applications under 37 CFR 1.53(b))

Transmitted herewith for filing is the patent application of:

**INVENTOR(S): Hans Heinrich HEIDTMANN, Rolf MUELLER and Hans-Harald
SEDLACEK**

**TITLE: NUCLEIC ACID CONSTRUCT FOR EXPRESSING ACTIVE SUBSTANCES WHICH
CAN BE ACTIVATED BY PROTEASES, AND PREPARATION AND USE**

In connection with this application, the following are enclosed:

APPLICATION ELEMENTS:

XX Specification - 59 TOTAL PAGES

(preferred arrangement:)

- Descriptive Title of the Invention
- Cross Reference to Related Applications
- Statement Regard Fed sponsored R&D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

XX Drawings - Total Sheets 3

XX Declaration and Power of Attorney - Total Sheets 2

 Newly executed (original or copy)

XX Copy from a prior application (37 CFR 1.63(d))

(relates to continuation/divisional boxes completed) - NOTE: Box below

 DELETION OF INVENTOR(S) - Signed statement attached deleting inventor(s)
named in the prior application, see 37 CFR 1.63(d) (2) and 1.33(b).

XX Incorporation By Reference (useable if copy of prior application
Declaration being submitted)

The entire disclosure of the prior application, from which a COPY of the
oath or declaration is supplied as noted above, is considered as being
part of the disclosure of the accompanying application and is hereby
incorporated by reference therein.

 Microfiche Computer Program (Appendix)

 Nucleotide and/or Amino Acid Sequence Submission (if applicable,
all necessary)

 Computer Readable Copy

 Paper Copy (identical to computer copy)

 Statement verifying identify of above copies

ACCOMPANYING APPLICATION PARTS

 Assignment Papers (cover sheet & document(s))

 37 CFR 3.73(b) Statement (when there is an assignee)

 English Translation Document (if applicable)

Information Disclosure Statement (IDS) with PTO-1449. Copies of IDS Citations
XX Preliminary Amendment
XX Return Receipt Postcard (MPEP 503)
 Small Entity Statement(s)
 Statement file in prior application, status still proper and desired.
 Certified Copy of Priority Document(s) with Claim of Priority
 (if foreign priority is claimed).
XX OTHER: Check for \$760.00

If a **CONTINUING APPLICATION**, check appropriate box and supply the requisite information:

 Continuation XX Divisional Continuation-in-part (CIP)
 of prior application Serial No. 09/008,308.

XX Amend the specification by inserting before the first line the following sentence: --This application is a continuation, XX divisional or continuation-in-part of application Serial No. 09/008,308, filed January 16, 1998.--

CORRESPONDENCE ADDRESS:

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FEE CALCULATIONS: (Small entity fees indicated in parentheses.)

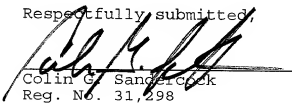
(1) For	(2) Number Filed	(3) Number Extra	(4) Rate	(5) Basic Fee \$760 (\$380)
Total Claims	4 - 20 =	0	x \$18 (x \$ 9)	0.00
Independent Claims	1 - 3 =	0	x \$78 (x \$39)	0.00
Multiple Dependent Claims			\$260 (\$130)	0.00
Assignment Recording Fee per property			\$40	0.00
			TOTAL FEE:	\$760.00

METHOD OF PAYMENT:

A check in the amount of the above TOTAL FEE is attached. If payment is enclosed, this amount is believed to be correct; however, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 19-0741.

Respectfully submitted,

Date: 2/24/99
Docket No.: 026083/0195


Colin G. Sandercock
Reg. No. 31,298

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 026083/0195

In re patent application of
Hans Heinrich HEIDTMANN et al.

Serial No.: Unassigned

Filed: February 4, 1999

For: NUCLEIC ACID CONSTRUCT FOR EXPRESSING ACTIVE
SUBSTANCES WHICH CAN BE ACTIVATED BY PROTEASES,
AND PREPARATION AND USE

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Prior to examination of the above-identified application, Applicants respectfully request that the following amendments be entered into the application:

IN THE CLAIMS:

Please cancel claims 1-18, 20, 22, and 24 without prejudice disclaimer.

Please amend the claims as follows:

19. (Amended) A method for the treatment or prophylaxis of tumors, leukemias, allergies, autoimmune diseases, infections, inflammations, transplant rejection reactions, thromboses, blood vessel occlusions, blood coagulation, blood circulation disturbances, injuries to tissues, or damage to the nervous system, comprising administering to a mammal an effective amount of [a polypeptide expressed by the nucleic acid construct of claim 1] said polypeptide of claim 25.

21. (Amended) A method for preparing [a polypeptide which is encoded by said nucleic acid

construct of claim 1] said polypeptide of claim 25,
comprising transducing a suitable cell with said
construct, expressing said polypeptide in said cell, and
isolating said expressed polypeptide.

25. (Amended) A [protein] polypeptide encoded by
[said] a nucleic acid construct [of claim 1] comprising
the following nucleic acid sequences in the following
order:

a) at least one promoter element operably
linked to;

b) at least one nucleic acid sequence which
encodes an active compound, wherein said active compound
is endogenous to mammals, operably linked to;

c) at least one nucleic acid sequence which
encodes an amino acid sequence cleavable specifically by
an enzyme which is released from a mammalian cell,
operably linked to;

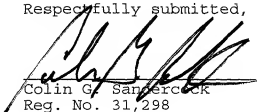
d) at least one DNA sequence which encodes
a polypeptide which is bound to said active compound by
said cleavable amino acid sequence and inhibits the
activity of said active compound, and wherein said nucleic
acid component c) does not naturally occur as operably
linking said nucleic acid sequence b) to said nucleic acid
d).

REMARKS

Entry of the foregoing amendments prior to examination is respectfully requested.

Respectfully submitted,

2/24/99


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5 **NUCLEIC ACID CONSTRUCT FOR EXPRESSING ACTIVE
SUBSTANCES WHICH CAN BE ACTIVATED BY PROTEASES,
AND PREPARATION AND USE**

Background of the Invention

The present invention relates to a nucleic acid
construct for expressing active substances which can be
10 activated by proteases and to its preparation and use.

Like inflamed areas, tumors are distinguished from
the surrounding normal tissue by a substantial increase
in the formation and secretion of proteases [Schmitt et
al., Fibrinol. 6, 3 (1992), Cottam et al., Int. J. Oncol.
15 2, 861 (1993), Tryggvason et al., Breast Cancer Res. And
Treatm. 24, 209 (1993), Leto et al., Anticancer Res. 12,
235 (1992), Hart, Fibrinol. 6, 11 (1992), Albini et al.,
J. Natl. Cancer Inst. 83, 735 (1991)]. Examples of these
proteases are plasminogen activators, cathepsins and
20 matrix metalloproteinases.

An essential function of these tumor proteases is to
dissolve the extracellular matrix to allow the tumor
cells to invade, and grow in an infiltrative manner in,
normal tissue. At the same time, these proteases protect
25 the tumor from the defence mechanisms of the body insofar
as the active compounds which are required for defence
are cleaved, and thereby inactivated, by the proteases
which are formed by the tumor. Thus, for example,
antibodies, cytokines and growth factors, complement
30 factors, coagulation factors and mediators are
inactivated by tumor proteases.

In the past, the aim was, therefore, to inhibit the
infiltrative growth and metastatic growth of tumors, and
inactivation of the defence mechanisms of the body, by
35 inhibiting the tumor cell proteases [Hocman, Int. J.
Biochem. 24, 1365 (1992), Troll et al., JNCI 73, 1245
(1984), Ray et al., Eur. Respir. 7, 2062 (1994), Koop et
al., Cancer Res. 54, 4791 (1994), Chiriri et al., Int. J.

Cancer 58, 460 (1994), Denhardt et al., 59, 329 (1993), Melchiori et al., Cancer Res. 52, 2353 (1992)]. However, particularly for stoichiometric and pharmacokinetic reasons, little success has previously been achieved in inhibiting tumor cell proteases.

An attempt was therefore made to use the tumor cell proteases to activate bacterial toxins such as Staphylococcus aureus α -hemolysin [Panchal et al., Nature Biotechn. 14, 852 (1996)]. For this, an amino acid sequence, i.e. XX-Arg-X, was inserted into positions 129 to 132 of the α -hemolysin and in this way inactive mutants were produced which are only cleaved, and thereby activated, by tumor proteases such as cathepsin B.

Based on these results, proimmunolysins were proposed [Panchal et al., Nature Biotechn. 14, 852 (1996)], which proimmunolysins comprise an antibody which is coupled to a Staphylococcus aureus α -hemolysin which can be activated by tumor proteases or to a sea anemone equinatoxin II, with the antibody determining the target cell specificity of the coupling product.

However, the proposed concept suffers from the following disadvantages in relation to its use in tumor therapy:

In the first place, the authors chose xenogeneic nonendogenous lysins and/or toxins which are immunogenic for the host organism (for example, patients) and as a result induce an immune reaction in the host organism, which immune reaction neutralizes and inactivates the antibody/toxin conjugate. In the second place, it is known [Sedlacek et al., Antibodies as Carriers of Cytotoxicity, Contrib. to Oncol. 43, Karger Verlag, Munich, 1992] that, due to their molecular size and to the rheological conditions at the tumor, tumor-specific antibodies and immunotoxins only accrue in very small quantities (0.01 - 0.001 % of the given antibody or immunotoxin/g of tumor) at the tumor and only penetrate

the tumor to an incomplete extent so that it is either not possible to destroy all the tumor cells or only possible to destroy a small portion of the cells of a tumor. Then again, the extent to which tumor antigens, against which the antibody is directed, are expressed usually differs between the individual tumor cells, and the variable, antigen-negative tumor cells readily evade the attack by the antibodies or the immunotoxins. In addition to this, antigens which are secreted by the tumor cells neutralize the antibodies at the periphery of the tumor (Sedlacek et al., Monoclonal Antibodies in Tumor Therapy, Contrib. to Oncol., Karger Verlag, 1988).

Consequently, there is still a great need for a target cell-specific therapy for tumors and inflammations.

Summary of the Invention

An object of the present invention is therefore to provide an active compound against tumors and inflammations, which active compound does not exhibit said disadvantages. The present invention therefore relates to a novel technique which uses the secretion of enzymes in tumors or areas of inflammation to achieve the local release of active compounds whose inactive precursors are expressed in tumor cells, tumor-associated cells or inflammatory cells.

One part of the subject-matter of the present invention is therefore a nucleic acid construct for expressing an active substance which is activated by an enzyme which is released from mammalian cells, which nucleic acid construct comprises the following components:

- a) at least one promoter element,
- b) at least one DNA sequence which encodes an active compound (protein B),

c) at least one DNA sequence which encodes an amino acid sequence (part structure C) which can be cleaved specifically by an enzyme which is released from a mammalian cell, and

5 d) at least one DNA sequence which encodes a peptide or protein (part structure D) which is bound to the active compound (protein B) by way of the cleavable amino acid sequence (part structure C) and inhibits the activity of the active compound (protein B).

10 Further objects of the instant invention are described as follows:

1. A nucleic acid construct for expressing an active substance which is activated by an enzyme which is released from mammalian cells, wherein the construct
15 comprises the following nucleic acid sequences in the following order:

a) at least one promoter element operably linked to;

20 b) at least one nucleic acid sequence which encodes an active compound, wherein the active compound is endogenous to mammals, operably linked to;

c) at least one nucleic acid sequence which encodes an amino acid sequence cleavable specifically by an enzyme which is released from a mammalian cell, operably
25 linked to;

d) at least one DNA sequence which encodes a polypeptide which is bound to the active compound by the cleavable amino acid sequence and inhibits the activity of the active compound, and wherein the nucleic acid
30 component c) does not naturally occur as operably linking the nucleic acid sequence b) to the nucleic acid d).

2. A nucleic acid construct as described in 1, wherein the enzyme is a protease.

3. A nucleic acid construct as described in 1,
35 wherein the enzyme is a prostate specific antigen, a

plasminogen activator, a cathepsin or a matrix metalloproteinase.

4. A nucleic acid construct as described in 1, wherein the mammalian cells are tumor cells, leukemia
5 cells, endothelial cells, macrophages, lymphocytes, muscle cells, epithelial cells, glia cells, synovial cells or virus-infected cells.

5. A nucleic acid construct as described in 1, wherein the nucleic acid construct further comprises a
10 nucleic acid sequence operably linked to the construct of 1, wherein the nucleic acid sequence encodes a ligand which binds the active compound to a target structure.

6. A nucleic acid construct as described in 1, wherein the nucleic acid sequences b) and d) of 1 encode
15 parts of a natural precursor of a protein active compound, wherein the nucleic acid sequence encoding the cleavage sequence naturally occurring between the nucleic acid sequences b) and d) has been replaced by the nucleic acid sequence c), which does not naturally occur between
20 the nucleic acid sequences b) and d).

7. A nucleic acid construct as described in 1, wherein the polypeptide encoded by the nucleic acid sequence d) is part of a natural precursor of a protein active compound.

25 8. A nucleic acid construct as described in 1, wherein the construct is operably inserted into a plasmid or a viral vector.

9. A nucleic acid construct as described in 1, wherein the nucleic acid sequence a) is a promoter
30 sequence which can be activated nonspecifically, cell-specifically, virus-specifically, metabolically, cell cycle-specifically or by tetracycline.

10. A nucleic acid construct as described in 1, wherein the nucleic acid sequence a) comprises at least
35 two identical or two different promoter sequences.

11. A nucleic acid construct as described 9, wherein the nucleic acid sequence a) is activated in endothelial cells, in cells adjoining activated endothelial cells, in muscle cells, in leukemia cells, in tumor cells, in glia cells, in lymphocytes, in macrophages or in synovial cells.

12. A nucleic acid construct as described in 1, wherein the active compound activates or inhibits a biological activation cascade or is an active component of this cascade, or activates or inhibits the coagulation system, activates fibrinolysis, activates the complement system or activates the kinin system, or is an enzyme which converts the inactive precursor of a pharmacological substance into the active substance, or which itself is a pharmacologically active substance.

13. A nucleic acid construct as described in 12, wherein the active compound is a coagulation factor which is selected from the group consisting of thrombin, factor Va, factor VIIa, factor IXa, factor Xa, TF coagulation-active fragments or factor XIIa; thrombin which is mutated in the region of the Arg-Thr cleavage site (amino acid position 327/328); a fibrinolytic protein which is selected from urokinase, tPA or functional hybrids thereof; a complement factor which is selected from C5, C3b or functional cleavage products thereof; an antithrombotic protein which is selected from protein C, C-1S inhibitor, α 1-antitrypsin, hirudin, AT-III, TFPI, PAI-1, PAI-2 or PAI-3; a kallikrein; a cytostatic, cytotoxic or inflammation-eliciting protein; an antiangiogenic protein; an immunomodulatory protein; an antiinflammatory protein; a protein which relieves damage to the nervous system; a protein which inhibits or neutralizes the neurotoxic effect of TNF α ; an angiogenesis-stimulating protein; a hypotensive protein; an antiviral protein; a cytokine; an interferon; a tumor necrosis factor; oncostatin M or LIF; a cytokine

receptor; the moiety of a cytokine receptor which is external to the cell; a cytokine antagonist; a growth factor; a growth factor receptor; the moiety of a growth factor receptor which is external to the cell; a chemokine; angiostatin; platelet factor 4; TIMP-1, TIMP-2 or TIMP-3; a nitroreductase; a β -glucuronidase; a carboxypeptidase; a β -lactamase; a cytosine deaminase; a catalase; a peroxidase; a phosphatase; an oxidase; kallikrein or an endothelial cell nitric oxide synthase.

10 14. A nucleic acid construct as described in 1, which further comprises a nucleic acid sequence b') which encodes a ligand which binds to a cell membrane receptor, a cell membrane antigen, a cell membrane-located adhesion molecule, or to the extracellular matrix or component thereof.

15 15. A nucleic acid construct as described in 14, wherein the ligand is an antibody or an antibody fragment which binds specifically to a cell membrane antigen or to an antigen on the extracellular matrix, or is a polypeptide which binds to receptor on the cell membrane
20 wherein the polypeptide is a growth factor, a cytokine, an interferon, a tumor necrosis factor, a chemokine, a receptor-binding part sequence of these ligands, a peptide hormone, angiotensin, kinin, folic acid, an adhesion molecule or the part sequence of the adhesion
25 molecule which binds to the corresponding adhesion molecule or to the extracellular matrix, an extracellular moiety of an Fc receptor, a glycoprotein of a virus, a part sequence of the glycoprotein which binds to these
30 cells, the transmembrane domain of a receptor or of a viral glycoprotein, or a glycopospholipid anchor.

35 16. A nucleic acid construct as described in 14, wherein the ligand binds to activated or proliferating endothelial cells, to tumor cells, to muscle cells, preferably smooth muscle cells, to fibroblasts, to macrophages, to lymphocytes, to liver cells, to kidney

cells, to synovial cells, to inflammatory cells, to virus-infected cells, to bronchial epithelial cells, to glia cells or to leukemia cells.

5 17. A nucleic acid construct as described in 14, wherein the construct comprises at least two identical or different nucleic acid sequences b)c)d) or b')b)c)d), which nucleic acid sequences are linked to each other by way of an internal ribosomal entry site.

10 18. A process for preparing a nucleic acid construct according to 1, which comprises operably linking the nucleic acid sequences of 1.

15 19. A method for the treatment or prophylaxis of tumors, leukemias, allergies, autoimmune diseases, infections, inflammations, transplant rejection reactions, thromboses, blood vessel occlusions, blood coagulation, blood circulation disturbances, injuries to tissues, or damage to the nervous system, comprising administering to a mammal an effective amount of a polypeptide expressed by the nucleic acid construct of 1.

20 20. A method for preparing a recombinantly altered cell, comprising transducing a suitable cell with the nucleic acid construct of 1.

25 21. A method for preparing a polypeptide which is encoded by the nucleic acid construct of 1, comprising transducing a suitable cell with the construct, expressing the polypeptide in the cell, and isolating the expressed polypeptide.

30 22. The method of 20, wherein the cell is an endothelial cell, a lymphocyte, a macrophage, a glia cell, a fibroblast, a liver cell, a kidney cell, a muscle cell, a cell of the bone or cartilage tissue, a synovial cell, a peritoneal cell, a skin cell, an epithelial cell, a leukemia cell or a tumor cell.

35 23. The method of 21, wherein the cell is an endothelial cell, a lymphocyte, a macrophage, a glia cell, a fibroblast, a liver cell, a kidney cell, a muscle

cell, a cell of the bone or cartilage tissue, a synovial cell, a peritoneal cell, a skin cell, an epithelial cell, a leukemia cell or a tumor cell.

24. A cell transduced with the nucleic acid construct of 1.

25. A protein encoded by the nucleic acid construct of 1.

The term "endogenous to mammals" as used to describe the active compound of the instant invention denotes a polypeptide that is naturally expressed in mammals or a derivative thereof as discussed herein.

The term "does not naturally occur" as used to describe the linking nucleic acid component c) denotes that the described component c) of the instant invention is not found in nature as operably linking components b) and d).

Brief Description of the Drawings

Figure 1 is a diagrammatic representation of a novel nucleic acid construct comprising components a), b), c) and d);

Figure 2 is a diagrammatic representation of a novel nucleic acid construct which has been enlarged by adding component b'); and

Figure 3 is diagrammatic representation of a nucleic acid construct for PSA-activatable factor X.

Detailed Description of the Preferred Embodiments

In their simplest form, the individual components can be arranged, for example, as shown in Figure 1. In this case, expression of a protein BCD, encoded by components b), c) and d), is induced by activating the promoter sequence [component a)]. The amino acid sequence C of the expression product is then cleaved by cellular enzymes, e.g. proteases, as a result of which protein B, which constitutes the active compound, is released.

Within the meaning of the present invention, proteases or enzymes are to be understood as being one or more proteases or enzymes.

5 In another embodiment, said enzyme is a protease, in particular a plasminogen activator, a cathepsin or a matrix metalloproteinase. Said mammalian cells are preferably tumor cells, leukemia cells, endothelial cells, macrophages, lymphocytes, muscle cells, epithelial cells, glia cells, synovial cells or virus-infected
10 cells.

Enzymes are preferably released, in an organism, by tumors and tumor cells and also by cells which are involved in an inflammatory process [Barrett et al., Mammalian Proteases, Academic Press, London 1980; Sedlacek and Möröy, Immune Reactions, Springer Verlag,
15 1995)].

According to the present invention, component c) is consequently selected such that the expressed protein, e.g. BCD, is preferably cleaved, in its part structure C,
20 by proteases which are formed in tumors or secreted by tumor cells or inflammatory cells. Examples of these proteases are plasminogen activators, such as plasminogen activator of the urokinase type or tissue plasminogen activator; cathepsins, such as cathepsin B, cathepsin D,
25 cathepsin L, cathepsin E or cathepsin H, or their precursors (procathepsins); matrix metalloproteinases (MMP), such as collagenases, for example of groups I, II, III, IV or V; stromelysin 1, stromelysin 2 or stromelysin 3; metrilysins; gelatinases, such as gelatinase A (MMP
30 2), and progelatinase B (MMP 9) and progelatinase A [Pappot et al., Lung Cancer 12, 1 (1995), Schmitt et al., Fibrinolysis 614, 3 (1992), Monsky et al., Cancer Biol. 4, 251 (1993), Rochefort et al., Medicine/Sciences 7, 30 (1991), Kao et al., 46, 1349 (1986), Fridman et al.,
35 Cancer Res. 55, 2548 (1995), Ray et al., Eur. Respir. J. 7, 2062 (1994), Cottam et al., Int. J. Oncol. 2, 861

(1993), Tryggvason et al., Breast Cancer Res. and Treatm. 24, 209 (1993)]; tumor cell surface proteases [surface-expressed proteases = seprase; Monsky et al., Cancer Res. 54, 5702 (1994)]; elastase [Kao et al., Cancer Res. 46, 1355 (1986)]; prostate-specific antigen [Lundwall, Biochem. Biophys. Res. Commun. 161, 1151 (1989), Riegman et al., Biochem. Biophys. Res. Commun. 159, 95 (1989)] or pancreatic trypsinogens [Miszuk-Jamska et al., FEBS Lett. 294, 175 (1991)].

In accordance with another embodiment of the present invention, the nucleotide sequence for component b) can be extended by the addition of a component b'). This component b') encodes a ligand (part structure B') which can bind the active compound to a target structure. Component b') is, for example, arranged as shown in Figure 2. Expression of the nucleic acid construct corresponding to Figure 2 results in a protein, i.e. B'BCD, which binds to a target structure by way of the ligand (part structure B'). The part structure C is then cleaved by an enzyme, for example, cellular proteases, thereby releasing the active compound, i.e. protein B'B.

In a particular embodiment, said protein B and the part structure D are parts of the natural precursors of protein active compounds, with the natural cleavage sequence, which connects the part structures B and D, having been replaced by the part structure C; in particular, said part structure D is the part structure of a natural precursor of a protein active compound.

The novel nucleic acid constructs are preferably composed of DNA. The term "nucleic acid constructs" is understood to mean artificial nucleic acid structures which can be transcribed in the target cells. They are preferably inserted into a vector, with plasmid vectors or viral vectors being particularly preferred.

Depending on the choice of the promoter element [component a)], the novel nucleic acid constructs express

a structural gene [components b) + c) + d) or b') + b) + c) + d)] either nonspecifically, cell-specifically, virus-specifically, under particular metabolic conditions, cell cycle-specifically or in the presence of tetracycline. At least two identical or different promoter elements can also be combined together for the purpose of modifying the expression of the structural gene depending on the choice of these promoter elements. Component a) is preferably activated in endothelial cells, in cells adjoining activated endothelial cells, in muscle cells, in leukemia cells, in tumor cells, in glia cells, in lymphocytes, in macrophages and/or in synovial cells.

The part structure B (protein B) of the protein encoded by the novel structural gene constitutes the actual novel active compound which is released or activated by cleavage of the part structure C and thereby converted from the inhibited state, e.g. as protein BCD or as protein B'BCD, into the active state, e.g. as protein B or as protein B'B.

According to the invention, this active compound can be an enzyme which activates or inhibits a biological activation cascade and/or is an active component of this cascade. Examples of biological activation cascades of this nature are the coagulation system, which can be activated or inhibited, fibrinolysis, which is preferably activated, the complement system, which is likewise preferably activated, or the kinin system, which is also preferably activated. The active compound can also be an enzyme which converts the inactive precursor of a pharmacological substance into the active substance or which itself is a pharmacologically active substance. Particular preference is given to an active compound (protein B) which is a coagulation factor which is selected from thrombin, factor Va, factor VIIa, factor IXa, factor Xa, TF coagulation-active fragments or factor

XIIa; thrombin which is mutated in the region of the Arg-Thr cleavage site (amino acid position 327/328); a fibrinolytic protein which is selected from urokinase, tPA or functional hybrids thereof; a complement factor which is selected from C3b or functional cleavage products thereof; an antithrombotic protein which is selected from protein C, C-1S inhibitor, α 1-antitrypsin, hirudin, AT-III, TFPI, PAI-1, PAI-2 or PAI-3; a kallikrein; a cytostatic, cytotoxic or inflammation-eliciting protein; an antiangiogenic protein; an immunomodulatory protein; an antiinflammatory protein; a protein which relieves damage to the nervous system; a protein which inhibits or neutralizes the neurotoxic effect of TNF α ; an angiogenesis-stimulating protein; a hypotensive protein; an antiviral protein; a cytokine; an interferon; a tumor necrosis factor; oncostatin M or LIF; a cytokine receptor; the moiety of a cytokine receptor which is external to the cell; a cytokine antagonist; a growth factor; a growth factor receptor; the moiety of a growth factor receptor which is external to the cell; a chemokine; angiostatin; platelet factor 4; TIMP 1, TIMP 2 or TIMP 3; a nitroreductase; a β -glucuronidase; a carboxypeptidase; a β -lactamase; a cytosine deaminase; a catalase; a peroxidase; a phosphatase; an oxidase; kallikrein or an endothelial cell nitric oxide synthase.

The part structure B' of the protein encoded by the novel structural gene constitutes the novel ligand for binding the active compound (protein B) to a target structure. A preferred target structure is the surface of cells, preferably a cell membrane receptor, a cell membrane antigen, a cell membrane-located adhesion molecule, or the extracellular matrix, for example of endothelial cells, in particular of activated or proliferating endothelial cells, tumor cells, muscle cells, in particular smooth muscle cells, fibroblasts, macrophages, lymphocytes, liver cells, kidney cells,

synovial cells, inflammatory cells, virus-infected cells, bronchial epithelial cells, glia cells, leukemia cells or cells of other tissues and organs. A particularly preferred target structure is the surface of activated and/or proliferating endothelial cells.

Another preferred target structure is constituted by components of the extracellular matrix, for example collagens [Prockop et al., Annu. Rev. Biochem. 64, 403 (1995), Wetzels et al., Am. J. Pathol. 139, 451 (1991)];
 10 ficolin [Ichijo et al., J. Biol. Chem. 268, 14505 (1993)]; sialoprotein [Bellahcene et al., Cancer Res. 54, 2823 (1994)]; laminin [von der Mark et al., Biochem. Biophys. Acta 823, 147 (1985); Hunt. Expl. Cell Biol. 57, 165 (1989)]; proteoglycans [Schmidtchen et al., Biomed.
 15 Chromatography 7, 48 (1993)] or tenascin [Oyama et al., Cancer Res. 51, 4876 (1991); Herlyn et al., Cancer Res. 51, 4853 (1991)].

The novel ligand (part structure B') can, for example, be an antibody or an antibody fragment, such as the epitope-binding moiety of an antibody, Fab, Fv, single-chain Fv or Fc, which binds specifically to a cell membrane antigen or to an antigen on the extracellular matrix, or another peptide or protein which binds to a receptor on the relevant cell membrane. These include,
 20 for example, growth factors, cytokines, interferons, tumor necrosis factor, chemokines, their receptor-binding part sequences, peptide hormones, angiotensin, kinin or folic acid. The ligand can also be an adhesion molecule or its adhesion sequence which binds to a corresponding
 30 molecule on the cell membrane or on the extracellular matrix, or the target cell-binding moiety, an extracellular moiety of an Fc receptor, a target cell-binding glycoprotein of a virus which has a tropism for selected cells, or a part sequence of the glycoprotein
 35 which binds to these cells, or a peptide by whose help the active compound is anchored in the cell membrane of

the cell which is expressing it. Examples of these anchoring peptides are the transmembrane domains of receptors or virus proteins or glycopospholipid anchors.

Component d) encodes a peptide (part structure D) which is bonded to protein B or protein B'B by way of part structure C and thereby inhibits the activity of protein B. Component d) can be any arbitrary nucleic acid sequence. Preferably, however, it is composed of nucleic acid sequences which encode endogenous peptides or proteins, in order to avoid or decrease the danger of an immune reaction. In another preferred embodiment, components b) and d) of the novel structural gene encode endogenous proteins or peptides.

A considerable number of protein active compounds occur in nature in the form of inactive precursors (protein BSD). A precursor of this type is activated by enzymes cleaving this precursor into a part structure which constitutes the actual protein active compound (protein B) and into an inactive part structure (part structure D). This precursor is cleaved at least one defined amino acid sequence, i.e. the so-called cleavage sequence (part structure S).

It is a particular part of the subject-matter of this invention that this cleavage sequence (part structure S) which occurs naturally in precursors of protein active compounds is replaced by the part structure C. This replacement is effected by the sequence encoding the part structure S being replaced by component c), encoding part structure C, in the nucleic acid sequence which encodes the natural precursor (protein BSD). After components a) and, where appropriate, b') have been added on, a novel nucleic acid construct is produced which comprises, for example, components a)b')b)c)d) or a)b)c)d), the part structure C of whose expression product, i.e. protein B'BCD or BCD, respectively, is cleaved by proteases which are formed in tumors or secreted by tumor cells or

inflammatory cells, such that the active compound, i.e. protein B'B or B, can be formed.

In another embodiment, the novel construct comprises at least two identical or different components b)c)d) and/or b')b)c)d), which components are linked to each other by way of a so-called internal ribosomal entry site (IRES).

Having been inserted into a nonviral vector or viral vector, the novel nucleic acid construct is generally, for the prophylaxis and/or therapy of disorders, administered locally or injected into the blood circulation. These disorders particularly include tumor diseases and inflammations. Such inflammations can be triggered, for example, by physicochemical damage, by an infection or by an immune reaction against endogenous or foreign tissue.

The present invention furthermore relates, therefore, to the use of a novel nucleic acid construct for preparing a drug for local or systemic administration for the prophylaxis and/or therapy of tumors, leukemias, allergies, autoimmune diseases, infections, inflammations, transplant rejection reactions, thromboses, blood vessel occlusions, blood coagulation and blood circulation disturbances, and injuries to tissues and/or damage to the nervous system.

The choice of the components of the novel nucleic acid construct depends on the disease which is to be treated by administering the nucleic acid construct and can be made as follows:

Promoter sequences [component a]]:

According to the present invention, particular preference is given, on the one hand, to promoter sequences [component a)] which are promoters and
 5 activator sequences which can be activated in an unrestricted manner, such as the promoter of RNA polymerase III, the promoter of RNA polymerase II, etc., the CMV promoter and CMV enhancer, or the SV40 promoter, and, on the other hand, to viral promoter and activator
 10 sequences, such as HBV, HCV, HSV, HPV, EBV, HTLV or HIV.

For example, in the case of the HIV promoter, the entire LTR sequence, including the TAR sequence [positions ≤ -453 to ≥ -80 , Rosen et al., Cell 41, 813 (1985)] can be used as a virus-specific promoter.

15 Metabolically activatable promoter and enhancer sequences, such as the hypoxia-inducible enhancer, promoters which can be activated in a cell cycle-specific manner, such as the promoters of the cdc25C gene, the cyclin A gene, the cdc2 gene, the Emyb gene, the DHFR
 20 gene or the E2F-1 gene, or tetracycline-activatable promoters, such as the tetracycline operator in combination with an appropriate repressor, are also particularly preferred as component a).

25 According to the present invention, nucleotide sequences which, after binding transcription factors, activate the transcription of a structural gene which adjoins them at the 3' end are also to be used as promoter sequences.

30 In addition, promoters which can be activated in a cell-specific manner are particularly preferred as component a). These promoters preferably include promoters or activator sequences composed of promoters or enhancers from those genes which preferably encode proteins in selected cells. For example, promoters for
 35 the following proteins are preferably to be used in the following cells:

Promoter and activator sequences which are activated in endothelial cells, such as brain-specific, endothelial glucose-I transporter, endoglin, VEGF receptor 2 (flt-1), VEGF receptor 2 (flk-1, KDR), tiel-1 or tiel-2, B61 receptor (Eck receptor), B61, endothelin, especially endothelin B and endothelin 1, endothelin receptors, in particular the endothelin B receptor, mannose 6-phosphate receptors, von Willebrand factor, IL-1 α , IL-1 β , IL-1 receptor, vascular cell adhesion molecule (VCAM 1) or synthetic activator sequences.

As an alternative to natural, endothelial cell-specific promoters, use can also be made of synthetic activator sequences which are composed of oligomerized binding sites for transcription factors which are preferentially or selectively active in endothelial cells. An example is transcription factor GATA 2, whose binding site in the endothelin 1 gene is 5'-TTATCT-3' [Lee et al., Biol. Chem. 266, 16188 (1991), Dormann et al., J. Biol. Chem. 267, 1279 (1992) and Wilson et al., Mol. Cell. Biol. 10, 4854 (1990)].

Promoters or activator sequences which are activated in cells in the vicinity of activated endothelial cells, in particular in smooth muscle cells, are present, for example, in the VEGF gene. The gene-regulatory sequences for the VEGF gene are the 5'-flanking region, the 3'-flanking region, the c-*Src* gene or the v-*Src* gene.

Steroid hormone receptors and their promoter elements, in particular the mouse mammary tumor virus promoter, or promoter elements of the gene encoding tropomyosin, α -actin, α -myosin, the receptor for PDGF, the receptor for FGF, MRF-4, phosphofructokinase A, phosphoglycerate mutase, troponin C, myogens, receptors for endothelin A, desmin or separate "artificial" promoters, are also suitable. Promoter elements to which the factors of the helix-loop-helix (HLH) family (MyoD, Myf 5, myogens and MRF4 [review in Olson and Klein, Genes

Dev. 8, 1 (1994)] can bind, as muscle-specific transcription factors, are likewise suitable. The muscle-specific transcription factors also include the zinc finger protein GATA-4 (Arcenci et al., Mol. Cell Biol. 13, 2235 (1993), Ip et al., Mol. Cell Biol. 14, 7517 (1994)] and the groups of the MEF transcription factors [Yu et al., Gene Dev. 6, 1783 (1992)].

The HLH proteins, and also GATA 4, exhibit a similar muscle-specific transcription not only with promoters from muscle-specific genes but also in a heterologous context, that is with "artificial" promoters. Examples of such artificial promoters are multiple copies of the (DNA) binding site for muscle-specific HLH proteins, such as the E box (myo D), e.g. 4× AGCAGGTGTTGGGAGGC, [Weintraub et al., PNAS 87, 5623 (1990)] or multiple copies of the DNA binding site for GATA 4 of the α-myosin heavy chain gene, e.g. 5'-GGCCGATGGCCAGATAGAGGGGGCCGATGGGCAGATAGAGG3' [Molkentin et al., Mol. Cell Biol. 14, 4947 (1994)].

Examples of promoters and activator sequences which are activated in leukemia cells are promoters for c-myc, HSP-70, bcl-1/cyclin D-1, bcl-2, IL-6, IL-10, TNFα, TNFβ, HOX-11, BCR-Abl, E2A-PBX-1 or PML-RATA.

Examples of promoters or activator sequences which are activated in tumor cells are promoter or activator sequences which interact with the transcription factors which are formed, or are active, in tumor cells. These preferred promoter or activator sequences include gene-regulatory sequences or elements from genes which encode proteins which are formed, in particular, in cancer cells or sarcoma cells. Thus, for example, the promoter of the N-CAM protein is used in the case of small-cell bronchial carcinomas, the promoter of the hepatitis growth factor receptor or of L-plastin is used in the case of ovarian carcinomas, and the promoter of L-plastin or of

polymorphic epithelial mucin (PEM) is used in the case of pancreatic carcinomas.

Promoters and activator sequences which are activated in glia cells are, in particular, the gene-regulatory sequences or elements from genes which encode, for example, the following proteins: the Schwann cell-specific protein periaxin, glutamine synthetase, glia cell-specific protein (glial fibrillary acid protein = GFAP), the glia cell protein S100b, IL-6 (CNTF), 5-HT receptors, TNF α , IL-10, insulin-like growth factor receptor I and II or VEGF. The gene-regulatory sequences for the VEGF gene have already been listed above.

Examples of promoters and activator sequences which are activated in lymphocytes and/or macrophages are the promoter and activator sequences of the gene encoding cytokines, cytokine receptors and adhesion molecules, and receptors for the Fc fragment of antibodies. Examples of these are: IL-1 receptor, IL-1 α , IL-1 β , IL-2, IL-2 receptor, IL-3, IL-3 receptor (α subunit), IL-3 receptor (β subunit), IL-4, IL-4 receptor, IL-5, IL-6, IL-6 receptor, interferon regulatory factor 1 (IRF-1), (the promoter of IRF-1 is activated to the same extent by IL-6 as by IFN γ or IFN β), IFN γ -responsive promoter, IL-7, IL-8, IL-10, IL-11, IFN γ , GM-CSF, GM-CSF receptor (α chain), IL-13, LIF, macrophage colony stimulating factor (M-CSF) receptor, type I and II macrophage scavenger receptors, MAC-1 (leukocyte function antigen), LFA-1 α (leukocyte function antigen) or p150,95 (leukocyte function antigen).

Examples of promoter and activator sequences which are activated in synovial cells are the promoter sequences for matrix metalloproteinases (MMP), for example for: MMP-1 (interstitial collagenase), or MMP-3 (stromelysin/transin). These also include the promoter sequences for tissue inhibitors of metalloproteinases (TIMP), for example TIMP-1, TIMP-2 and TIMP-3.

According to the present invention, several of the promoter sequences which have been listed by way of example can be combined with each other in order to achieve the highest possible target cell specificity in the expression of the novel nucleic acid construct. Two identical promoters can also be combined. Several promoter sequences can be combined, for example, using chimeric promoters or hybrid promoters. A chimeric promoter is the combination of an upstream activator sequence, which can be activated cell-specifically, metabolically or virus-specifically, with a downstream promoter module which binds the transcription factors of the CDF and CHF families or the E2F and CHF families and can thereby inhibit activation of the upstream activator sequence in the G0 and G1 phases of the cell cycle (Lucibello et al., EMBO J. 14, 132 (1994)).

In the case of hybrid promoters, the TATA box of a promoter is, for example, mutated, with this mutation being compensated for by a corresponding mutation in the gene for a TATA-binding protein, and this TATA-binding protein being under the control of another promoter.

Nucleic acid sequence [component b')], which encodes a ligand (part structure B'):

According to the present invention, the ligand is a substance which binds a membrane antigen to a receptor or to an adhesion molecule on the target cell or which is integrated in the cell membrane and/or binds to the extracellular matrix. Reviews of the important cytokines and growth factors and their receptors, adhesion molecules and extracellular matrix proteins are provided by Ayad et al., The Extracellular Matrix, Academic Press 1994; Callard et al., The Cytokine, Academic Press 1994; Pigott et al., The Adhesion Molecule, Academic Press 1994, and Barclay et al., The Leucocyte Antigen, Academic Press 1994.

Examples of substances which bind to receptors are growth factors, such as VEGF, PDGF, EGF, TGF α , TGF β , KGF, SDGF, FGF, IGF, HGF, NGF, BDNF, neurotrophins, BMF, bombesin, M-CSF, thrombopoietin, erythropoietin, SCF, 5 SDGF, oncostatin, PDEGF or endothelin-1, cytokines, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, interferons α , β and γ , tumor necrosis factors TNF α and TNF β , chemokines, such as RANTES, MCAF, MIP-1 α or MIP-1 β , NAP 10 or β -thromboglobulin, peptide hormones, such as SRH, SIH or STH, MRH or MSH, PRH, PIH or prolactin, LH-RH, FSH-RH, LH/ICSH or FSH, TRH or TSH, CRH or ACTH, angiotensin, kinins, homologs or analogs thereof, or vitamins, such as folic acid.

15 According to the present invention, the ligand can also be an adhesion molecule, a part of an adhesion molecule or an analog of an adhesion molecule which binds to a corresponding adhesion molecule which is located in the cell membrane or to another specific binding 20 structure for an adhesion molecule on the target cell or in the extracellular matrix.

Examples of such adhesion molecules which are capable of functioning as ligands are Lewis X (for GMP-140), S Lewis X (for ELAM-1), LFA-1 (for ICAM-1 and ICAM-2), MAC- 25 1 (for ICAM-1), VLA-4 (for VCAM-1), PECAM (for PECAM), vitronectin (for the vitronectin receptor), GMP-140 (for Lewis X), S Lewis X (for ELAM-1), ICAM-1, ICAM-2 (for LFA-1 and MAC-1), VCAM-1 (for VLA-4), fibronectin (for VLA-4), laminin (for VLA-6), laminin (for VLA-1, VLA-2 30 and VLA-3), fibrinogen (for GPIIb-IIIa), B7 (for CD28), CD28 (for B7), CD40 (for CD40L) or CD40L (for CD40).

According to the present invention, the ligand can also be the extracellular moiety of an Fc receptor [Dougherty et al., Transfusion Science 17, 121 (1996)]. 35 Furthermore, the ligand can also be an antibody molecule or the epitope-binding moiety of an antibody molecule.

The murine monoclonal antibodies should preferably be employed in humanized form. The humanization is effected in the manner described by Winter et al. Nature 349, 293 (1991) and Hoogenbooms et al. Rev. Tr. Transfus. Hemobiol. 36, 19 (1993).

Recombinant antibody fragments are either prepared directly from existing hybridomas or are isolated from libraries of murine or human antibody fragments [Winter et al., Annu. Rev. Immunol. 12, 433 (1994)] using the phage-display technique [Smith, Science 228, 1315 (1985)]. The antibody fragments are then employed directly, at the genetic level, for further manipulations, e.g. for fusion with other proteins.

In order to prepare recombinant antibody fragments from hybridomas, the genetic information which encodes the antigen-binding domains (VH and VL) of the antibodies is obtained by isolating the mRNA, reverse-transcribing the RNA into cDNA and then amplifying the cDNA by means of the polymerase chain reaction [Saiki et al., Science 230, 1350 (1985)] and using oligonucleotides which are complementary to the 5' and 3' ends of the variable fragments [Orlandi et al., 1989]. The VH and VL fragments are then cloned into bacterial expression vectors, for example in the form of Fv fragments [Skerra & Plückthun, Science 240, 1038 (1988)], single-chain Fv fragments (scFv) [Bird et al., Science 242, 423 (1988), Huston et al., PNAS-USA 85, 5879 (1988)] or as Fab fragments [Better et al., Science 240, 1041 (1988)].

The phage-display technique can also be used to isolate new antibody fragments directly from antibody libraries (immune libraries or naive libraries) of murine or human origin. In the phage-display of antibody fragments, the antigen-binding domains are cloned, as protein fusions with the coat protein g3P of filamentous bacteriophages, either into the phage genome [McCafferty et al., Nature 348, 552 (1990)] or into phagemid vectors

[Breitling et al., Gene 104, 147 (1991)] in the form of scFv fragments [McCafferty et al., Nature 348, 552 (1990)] or as Fab fragments [Hoogenboom et al., Nucl. Acid Res. 19, 4133 (1991), Barbas et al., PNAS-USA 88, 7978 (1991)]. Antigen-binding phages are selected on antigen-loaded plastic vessels (panning) [Marks et al., J. Mol. Biol. 222, 581 (1991)], on antigen-conjugated, paramagnetic beads [Hawkins et al., J. Mol. Biol. 226, 889 (1992)] or by binding to cell surfaces [Marks et al., Bio/Technol. 11, 1145 (1993)].

Immune libraries are prepared by subjecting the variable antibody fragments from the B lymphocytes of immunized animals [Sastry et al., PNAS-USA 86, 5728 (1989), Ward et al., Nature 341, 544 (1989), Clackson et al., Nature 352, 624 (1991)] or patients [Mullinax et al., PNAS-USA, 87, 8095 (1990), Barbas et al., PNAS-USA, 88, 7978 (1991)] to PCR amplification. For this, use is made of combinations of oligonucleotides which are specific for murine [Orlandi et al., PNAS-USA, 86, 3833 (1989), Sastry et al., PNAS-USA, 86, 5728 (1989)] or human immunoglobulin genes [Larrick et al., BBRC 160, 1250 (1989)] or for the human immunoglobulin gene families [Marks et al., Eur. J. Immunol. 21, 985 (1991)].

Naive libraries can be prepared, for example, using nonimmunized donors as the source of the immunoglobulin genes [Marks et al., J. Mol. Biol. 222, 581 (1991)]. Alternatively, immunoglobulin germ line genes can be used to prepare semisynthetic antibody repertoires, with the complementarity-determining region 3 of the variable fragments being amplified by PCR using degenerate primers [Hoogenboom & Winter, J. Mol. biol. 227, 381 (1992), Barbas et al., PNAS-USA, 89, 4457 (1992), Nissim et al., EMBO J. 13, 692 (1994), Griffiths et al., EMBO J. 13, 3245 (1994)]. As compared with immune libraries, these so-called single-pot libraries have the advantage that antibody fragments against a large number of antigens can

be isolated from one single library [Nissim et al., EMBO J, 13, 692 (1994)].

5 The phage-display technique can be used to increase the affinity of antibody fragments still further, with new libraries being prepared from already existing antibody fragments by random [Hawkins et al., J. Mol. Biol. 226, 889 (1992), Gram et al., PNAS-USA, 89, 3576 (1992)], codon-based [Glaser et al., J. Immunol. 149, 3903 (1992)] or site-directed mutagenesis [Balint & 10 Larrick, Gene 137, 109 (1993)], by shuffling the chains of individual domains with those of fragments from naive repertoires [Marks et al., Bio/Technol 10, 779 (1992)] or by using bacterial mutator strains [Low et al., J. Mol. Biol. 26, 359 (1996)], and antibody fragments having 15 improved properties being isolated by reselecting under stringent conditions [Hawkins et al., J. Mol. Biol. 226, 889 (1992)]. In addition, murine antibody fragments can be humanized by a stepwise replacement of one of the variable domains with a human repertoire and then 20 selecting with the original antigen (guided selection) [Jespers et al., Bio/Technol, 12, 889 (1994)]. Alternatively, murine antibodies are humanized by specifically replacing the hypervariable regions of human antibodies with the corresponding regions of the original 25 murine antibody [Jones et al., Nature 321, 522 (1987)].

According to the present invention, the ligand can also be the nucleotide sequence encoding a coat protein, or a part of a coat protein, of viruses which specifically bind to selected cells by way of their coat 30 protein.

The ligand can also be a peptide, with whose help the active compound (protein B) is anchored in the cell membrane of the expressing cells. These anchoring peptides include the transmembrane domains of cell 35 membrane-located receptors or of virus proteins, such as the transmembrane sequence of human macrophage colony-

stimulating factor [DNA position \leq 1485 to \geq 1554; Cosman et al., Behring Inst. Mitt. 83, 15 (1988)] or the DNA sequence for the signal and transmembrane regions of human respiratory syncytial virus (RSV) glycoprotein G [amino acids 1 to 63 or their part sequences, amino acids 38 to 63; Vijaya et al., Mol. Cell Biol. 8, 1709 (1988); Lichtenstein et al., J. Gen. Virol. 77, 109 (1996)] or the DNA sequence for the signal and transmembrane region of influenza virus neuraminidase [amino acids 7 to 35 or the part sequence of amino acids 7 to 27, Brown et al., J. Virol. 62; 3824 (1988)].

However, the nucleotide sequence for a glycopospholipid anchor [review of glycopospholipid-anchored membrane proteins in Ferguson et al., (Ann. Rev. Biochem. 57, 285 (1988))] can also be inserted for the purpose of anchoring the active compound in the cell membrane of the transduced cells which form the active compound. Glycopospholipid anchors have been described, for example, for CEA [DNA position $<$ 893 to $>$ 1079; Berling et al., Cancer Res. 50 6534 (1990)], for N-CAM [Cunningham et al., Science 236, 799 (1987)] and for other membrane proteins such as Thy-1 [Clissold, Biochem. J. 281, 129 (1992)] or CD16 [Selvaray et al., Nature 333, 565 (1988)].

The choice of the ligand depends, first and foremost, on the target cell which is to be transduced with the nucleic acid construct. Ligands for activated endothelial cells are examples of this. Within the meaning of the invention, these ligands include antibodies or antibody fragments which are directed against membrane structures of endothelial cells, as have been described, for example, by Burrows et al. Pharmac. Ther. 64, 155 (1994), Hughes et al., Cancer Res. 49, 6214 (1989) and Maruyama et al., PNAS-USA 87, 5744 (1990). In particular, these antibodies include antibodies against actin, angiotensin II receptors, antibodies against

receptors for growth factors such as VEGF, FGF, PDGF or EGF, and antibodies against adhesion molecules, for example against the vitronectin receptor or ICAM 3.

5 The ligands furthermore include all active compounds which bind to membrane structures or membrane receptors on endothelial cells. Examples of these are IL-1 or growth factors, or their fragments or part sequences thereof, which bind to receptors which are expressed in endothelial cells, for example PDGF, bFGF, VEGF or TGF β
10 [Pusztain et al., J. Pathol. 169, 191 (1993)].

The ligands furthermore include adhesion molecules which bind to activated and/or proliferating endothelial cells. Adhesion molecules of this nature, such as Slex, LFA-1, MAC-1, LECAM-1, VLA-4 or vitronectin, have already
15 been described [Augustin-Voss et al., J. Cell Biol. 119, 483 (1992), Pauli et al., Cancer Metast. Rev. 9, 175 (1990), Honn et al., Cancer Metast. Rev. 11, 353 (1992), Pigott et al., The Adhesion Molecule, Academic Press (1994)].

20 The ligands within the meaning of this invention also include, in particular, glycoproteins from the coats of viruses which have a tropism for endothelial cells. Examples of these viruses are filoviruses, such as Marburg virus with its coat proteins GP (glycoprotein)
25 and sGP (second glycoprotein) or Ebola virus, in each case with its coat proteins GP and sG, cytomegalovirus, particularly with its gB protein, herpes simplex virus type I, HIV-1 virus, measles virus, Hantaan virus, alphaviruses, such as Semliki forest virus, epidemic
30 hemorrhagic fever virus, polio virus or enteroviruses, such as ECHO 9, ECHO 12 and Coxsackie B3.

Antibodies or antibody fragments which are directed against membrane structures of muscle cells, in particular of smooth muscle cells, are examples of
35 ligands for muscle cells. Examples of antibodies of this nature are antibody 10F3, antibodies against actin,

antibodies against angiotensin II receptors, antibodies against receptors for growth factors or antibodies, for example, against EGF receptors, against PDGF receptors or against FGF receptors, or antibodies against endothelin A receptors.

The ligands furthermore include nucleotide sequences for active substances which bind to membrane structures or membrane receptors on muscle cells [Pusztai et al., J. Pathol. 169, 191 (1993), Harris, Curr. Opin. Biotechnol. 2, 260 (1991)]. Examples of these ligands are growth factors, or their fragments or part sequences thereof, which bind to receptors which are expressed in smooth muscle cells, for example PDGF, EGF, TGF β , TGF α , FGF or endothelin A.

The ligands also include glycoproteins from the coats of those viruses which have a tropism for muscle cells. An example of these viruses is cytomegalovirus [Speir et al., Science 265, 391 (1994)].

Examples of ligands for activated macrophages and/or activated lymphocytes are, in addition, nucleotide sequences which encode substances which bind specifically to the surface of immune cells. These substances include antibodies or antibody fragments which are directed against membrane structures of immune cells, as have been described, for example, by Powelson et al., Biotech. Adv. 11, 725 (1993) and Barclay et al., The Leucocyte Antigen, Academic Press (1994). The ligands also include monoclonal or polyclonal antibodies or antibody fragments which bind, by their antigen-binding variable moiety, to Fc γ , Fc ϵ or Fc μ receptors of immune cells [Rojanasakul et al., Pharm. Res. 11, 1731 (1994)]. They furthermore include the Fc fragment of human monoclonal or polyclonal immunoglobulin.

The ligands furthermore include all substances which bind to membrane receptors on the surface of immune cells. These substances include cytokines, such as IL-1,

IL-2, IL-3, IL-4, IL-6, IL-10, TNF α , GM-CSF and M-CSF, and also growth factors, such as EGF, TGF, FGF, IGF or PDGF, or their fragments or part sequences thereof, which bind to receptors which are expressed in immune cells
 5 [Callard et al., The Cytokine, Academic Press (1994)]. The ligands also include adhesion molecules and other ligands which bind to cell membrane structures on macrophages, and in spleen, liver, lung and other tissues [Pigott et al., The Adhesion Molecule, Academic Press
 10 (1994), Perales et al., Eur. J. Biochem. 226, 255 (1994)].

The ligands within the meaning of this invention also include glycoproteins from the coats of those viruses which have a tropism for lymphocytes and/or macrophages.
 15 Examples of these macrophage-infecting viruses are HIV-1, in particular those strains having mutations in the V3 region of gp120 which result in increased binding to macrophages, HIV-2, Hantaviruses, for example Punmalavirus, cytomegalovirus, respiratory syncytial
 20 virus, herpes simplex virus or filoviruses.

Examples of lymphocyte-infecting viruses are varicella zoster virus (VZV), since VZV infects T cells in particular, herpesvirus 6 (HHV 6), since HHV 6 likewise infects T cells in particular, rabies virus, since rabies virus coat protein binds to TH2 cells in particular, HIV-1, since glycoprotein gp120 binds preferably to the CD4 molecule of T cells, HTLV-II, since HTLV-II infects B cells in particular, HTLV-I, since HTLV-I infects T cells in particular, influenza C
 25 viruses, since influenza C viruses bind to N-acetyl-9- β -acetylneuraminic acid (Neu 5,9 Ac), which preferentially occurs on B lymphocytes and to a lesser extent, or not at all, on T lymphocytes, by way of the hemagglutinin-esterase fusion (HEF) protein, influenza C viruses having
 30 a mutation in nucleotide position 872, which encodes position 284 of the amino acid sequence of the HEF, for
 35

example with the threonine being replaced with isoleucine, since the surface protein HEF which possesses this mutation has a markedly stronger affinity for the N-acetyl-9-O-acetylneuraminic acid receptor than does the wild-type virus, cleavage products of the influenza C virus HEF which contain the structure for binding to N-acetyl-9- β -acetylneuraminic acid. This binding structure is defined by the catalytic triad serine 71, histidine 368 or 369 and aspartic acid 261, Epstein-Barr virus, since EBV infects B cells in particular, herpes simplex virus 2, since HSV-2 infects T cells in particular, or measles virus.

Examples of ligands for synovial cells and inflammatory cells which are to be mentioned are nucleic acid sequences which encode antibodies or antibody fragments which bind, by their variable domains, to membrane structures of synovial cells or inflammatory cells. Examples of these membrane structures are vimentin [Miettinen et al., Am. J. Pathol. 117, 18 (1984)], fibronectin [Wojciak et al., Clin. Exp. Immunol. 93, 108 (1993)] or Fc receptors. These ligands also include antibodies or antibody fragments which bind to the Fc receptor by their constant domains [Rojanasakul et al., Pharm. Res. 11, 1731 (1994)].

These ligands furthermore include all active compounds which bind to membrane structures or membrane receptors on synovial cells. Examples of these are cytokines or growth factors, or their fragments or part sequences thereof, which bind to receptors which are expressed by synovial cells, for example IL-1-RA, TNF α , IL-4, IL-6, IL-10, IGF or TGF β [Callard et al., The Cytokine, Academic Press (1994)].

Examples of ligands for virus-infected cells which are to be mentioned are nucleic acid constructs which encode antibodies or antibody fragments which are directed against the viral antigens which are located on

the cell membrane of virus-infected cells. Antibodies of this nature are directed, for example, against antigens of HBV, HCV, HSV, HPV, HIV, EBV or HTLV.

Examples of ligands for liver cells and other tissue cells are all substances which bind to membrane structures or membrane receptors on the surface of liver cells. Examples of these are growth factors, such as cytokines, EGF, TGF, FGF or PDGF, or their fragments or part sequences thereof, which bind to receptors which are expressed in cells of this nature.

These ligands furthermore include ligands which bind to cell membrane structures which are selective for particular tissues. Examples are:

	Ligand	Tissue cells
Transferrin receptor	Transferrin	Liver, other tissue cells
Insulin receptor	Insulin	Liver, other tissue cells
Fcγ receptors	Immunoglobulin G	Reticuloendothelial system, other tissue cells

These ligands and membrane structures are reviewed in Perales et al., Eur. J. Biochem. 226, 255 (1994).

The ligands particularly include glycoproteins from the coats of viruses which have a tropism for selected cells, such as for bronchial epithelial cells (respiratory syncytial virus), liver cells (hepatitis C virus), filoviruses, Marburg virus by way of the asialoglycoprotein receptor of liver cells, hepatitis B virus, with liver cells preferably binding to the preS2 and preS1 domains of HBV by way of the asialoglycoprotein receptor, hepatitis D virus, liver-sinusoidal cells, and

hepatitis B virus, with HBV being bound by way of fibronectin.

5 Examples of ligands for glia cells are nucleic acid sequences which encode antibodies or antibody fragments which are directed against membrane structures of glia cells, as have been reported, for example, by Mirsky et al. [Cell and Tissue Res. 240, 723 (1985)], Coakham et al. [Prog. Exp. Tumor Res. 29, 57 (1985)] and McKeever et al. [Neurobiol. 6, 119 (1991)]. These membrane
10 structures furthermore include neural adhesion molecules such as N-CAM, in particular its polypeptide chain C [Nybroe et al., J. Cell Biol. 101, 2310 (1985)]. These ligands furthermore include all active compounds which bind to membrane structures or membrane receptors on glia
15 cells. Examples of these active compounds are insulin and insulin-like growth factor, and those fragments of these growth factors which bind to the relevant membrane receptors.

20 The ligands within the meaning of the invention additionally include nucleic acid sequences which encode glycoproteins of the coats of those viruses which have a tropism for glia cells.

 These viruses include, for example, HIV-1 subtype JRF1 or herpes simplex virus I.

25 Examples of ligands for leukemia cells include nucleic acid constructs which encode antibodies or antibody fragments which are directed against membrane structures of leukemia cells. A large number of monoclonal antibodies of this nature have already been
30 described for diagnostic and therapeutic procedures [Kristensen, Danish Medical Bulletin 41, 52 (1994); Schranz, Therapia Hungarica 38, 3 (1990); Drexler et al., Leuk. Res. 10, 279 (1986); Naeim, Dis. Markers 7, 1 (1989); Stickney et al., Curr. Opin. Oncol. 4, 847
35 (1992); Drexler et al., Blut 57, 327 (1988); Freedman et al., Cancer Invest. 9, 69 (1991)]. Depending on the type

of leukemia, monoclonal antibodies, or their antigen-binding antibody fragments, of the following specificity are, for example, suitable as ligands:

5 AML cells having the membrane antigens CD13, CD14, CD15, CD33, CAMAL and sialosyl-Le; B-CLL cells having the membrane antigens CD5, CD1c and CD23, and also idiotypes and isotypes of the membrane immunoglobulins; T-CLL cells having the membrane antigens CD33, M38, IL-2 receptors and T cell receptors; and ALL cells having the membrane
10 antigens CALLA and CD19, and also non-Hodgkin's lymphoma.

The ligands furthermore include all active compounds which bind to membrane structures or membrane receptors of leukemia cells. Examples of these are growth factors, or their fragments or part sequences thereof, which bind
15 to receptors which are expressed in leukemia cells.

Growth factors of this nature have already been described [reviews in Cross et al., Cell 64, 271 (1991); Aulitzky et al., Drugs 48, 667 (1994); Moore, Clin. Cancer Res. 1, 3 (1995); Van Kooten et al., Leuk. Lymph.
20 12, 27 (1993)]. For example, they include IFN α , in the case of non-Hodgkin's lymphomas, IL-2, particularly in the case of T cell leukemias, FGF in the case of T cell, monocytic, myeloid, erythrocytic and megakaryoblastic leukemias, TGF β in the case of leukemias, or retinoids,
25 e.g. retinoic acid, in the case of acute promyelocytic leukemia.

Examples of ligands for tumor cells include nucleic acid sequences which encode antibodies, and fragments of these antibodies, which are directed against membrane
30 structures on tumor cells. Antibodies of this nature have been reviewed, for example, by Sedlacek et al., Contrib. to Oncol. 32, Karger Verlag, Munich (1988) and Contrib. to Oncol. 43, Karger Verlag, Munich (1992).

Other examples are antibodies against sialyl Lewis, peptides on tumors which are recognized by T cells,
35 proteins expressed by oncogenes, gangliosides such as

GD3, GD2, GM2, 9-O-acetyl-GD3 and fucosyl-GM1, blood group antigens and their precursors, antigens on polymorphic epithelial mucine or antigens on heat shock proteins.

5 Nucleic acid sequence [component b)] which encodes an active compound (protein B):

10 The active compound (protein B) according to the present invention can be a substance which, for example, intervenes in a biological activation cascade and/or is an active component of this cascade. These substances include active compounds which activate the coagulation cascade, for example thrombin [MacGillivray et al., Ann. N.Y. Acad. Sci. 485, 73 (1986)], thrombin which is mutated in the region of the Arg-Thr cleavage site (amino acid position 327/328), factor Va [Cripe et al., Biochem. 31, 3777 (1992), Jenny et al., PNAS-USA 84, 4846 (1987)], factor VIIa [O'Hara et al., PNAS-USA 84, 5158 (1987)], factor IXa [Yoshitake et al., Biochem. 24, 3736 (1985)], factor Xa [Messier et al., Gene 99, 291 (1991)] or tissue factor and coagulation-active fragments thereof [Morrissey et al., Cell 50, 29 (1987); Scarpati et al., Biochem. 26, 5234 (1987); Spicer et al., PNAS-USA 84, 5148 (1987); Rehemtulla et al., Thromb. Haemost. 65, 521 (1991)] or which inhibit the coagulation cascade or which activate fibrinolysis, for example the plasminogen activator inhibitors PAI-1, PAI-2 and PAI-3, hirudin, protein C, serine proteinase inhibitors, such as C-1S inhibitor, α 1-antitrypsin or antithrombin III, tissue factor pathway inhibitor (TFPI), plasminogen activators such as urokinase, tissue plasminogen activator (tPA), or hybrids thereof, or which activate the complement cascade, for example cobra venom factor (CVF) or part sequences of CVF which correspond functionally to human complement factor C3b, i.e. which are able to bind to complement factor B and which, after having been cleaved

by factor D, constitute a C3 convertase (the DNA sequence for CVF and its part sequences were described by Fritzinger et al., Proc. Natl. Acad. Sci. USA 91, 12775 (1994)), human complement factor C3b (the DNA sequence for C3 and its part sequences were published by De Bruijn et al., Proc. Natl. Acad. Sci. USA 82, 708 (1985), cleavage products of human complement factor C3 which resemble CVF functionally and structurally (such cleavage products have been described by O'Keefe et al., J. Biol. Chem. 263, 12690 (1988) or which activate the kinin system, the complement system and/or the coagulation system, for example activated Hagemann factor (F XIIa) [Shibuya et al., Biochem. Biophys. Acta 1206, 63 (1994), Que et al., Biochem. 25, 1525 (1986), Tripodi et al., Nucl. Acid Res. 14, 3146 (1986)] or kallikrein [Chen et al., Biochem. J. 307, 481 (1995), Fukushima et al., Biochem. 24, 8037 (1985)].

The active compound (protein B) can also be a cytostatic, cytotoxic or inflammation-eliciting protein, such as perforin, granzyme, cytokines, such as IL-1, IL-2, IL-4, IL-12, IL-3, IL-5, human leukemia inhibitory factor (LIF), IL-7, IL-11, IL-13, GM-CSF, G-CSF or M-CSF, interferons, such as IFN α , IFN β or IFN γ , TNF, such as TNF α or TNF β , oncostatin M, sphingomyelinase [Jarvis et al., PNAS USA 91, 73 (1994)], magainin and magainin derivatives [Cruciani et al., PNAS USA 88, 3792 (1991)]; Jacob et al., Ciba Found. symp. 186, 197 (1994); Peck-Miller et al., Cancer Chemother. Pharmac. 32, 109 (1993)] or chemokines, such as RANTES (MCP-2), monocyte chemotactic and activating factor (MCAF), IL-8, macrophage inflammatory protein 1 (MIP-1 α or MIP-1 β) or neutrophil activating protein 2 (NAP-2).

The active compound (protein B) can also be an antiangiogenic protein, such as angiostatin, interferons, such as IFN α , IFN β or IFN γ , platelet factor 4, IL-12, TIMP-1, TIMP-2 or TIMP-3.

The active compound (protein B) can also be an enzyme which is able to convert an inactive precursor of a pharmacological active substance, for example a cytostatic agent, into the active substance itself.

5 Examples of such active compounds are bacterial nitroreductase, bacterial β -glucuronidase, plant β -glucuronidase derived from *Secale cereale*, human β -glucuronidase, human carboxypeptidase (CB), e.g. mast cell CB-A or pancreas CB-B, or bacterial

10 carboxypeptidase, bacterial β -lactamase, bacterial cytosine deaminase, human catalase or peroxidase, phosphatase, in particular human alkaline phosphatase or human acid prostate phosphatase, type 5 acid phosphatase, oxidase, in particular human lysyl oxidase or human acid

15 D-aminooxidase, peroxidase, in particular human glutathione peroxidase, human eosinophilic peroxidase or human thyroid peroxidase.

The active compound (protein B) can also be a protein which affects the immune system, for example a protein

20 having an antiallergic effect, such as $\text{IFN}\beta$, $\text{IFN}\gamma$, IL-10, soluble IL-4 receptors, IL-12 or $\text{TGF}\beta$, or a protein which can prevent the rejection of transplanted organs, such as IL-10, $\text{TGF}\beta$, soluble IL-1 receptors, soluble IL-2 receptors, IL-2 receptor antagonists or soluble IL-6

25 receptors, or a protein for the therapy of antibody-mediated autoimmune diseases, for example $\text{TGF}\beta$, $\text{IFN}\alpha$, $\text{IFN}\beta$, $\text{IFN}\gamma$, IL-12, soluble IL-4 receptors or soluble IL-6 receptors, or a protein for the therapy of cell-mediated autoimmune diseases, for example IL-6, IL-9, IL-10, IL-

30 13, $\text{TNF}\alpha$, IL-4 or $\text{TNF}\beta$, or a protein for the therapy of arthritis. According to the present invention, structural genes can also be selected whose expressed protein directly or indirectly inhibits inflammation, for example in a joint, and/or promotes the reconstitution of

35 extracellular matrix (cartilage and connective tissue) in the joint. These expressed proteins include, for

example, IL-1 receptor antagonists (IL-1-RA), since IL-1-RA inhibits the binding of IL-1 α and IL-1 β , soluble IL-1 receptor, since soluble IL-1 receptor binds and inactivates IL-1, IL-6, since IL-6 increases secretion of TIMP and superoxides and decreases secretion of IL-1 and TNF α by synovial cells and chondrocytes, soluble TNF receptor, since soluble TNF receptor binds and activates TNF, IL-4, since IL-4 inhibits the formation and secretion of IL-1, TNF α and MMP, IL-10, since IL-10 inhibits the formation and secretion of IL-1, TNF α and MMP and increases the secretion of TIMP, insulin-like growth factor (IGF-1), since IGF-1 stimulates the synthesis of extracellular matrix, TGF β , especially TGF β 1 and TGF β 2, since TGF β stimulates the synthesis of extracellular matrix superoxide dismutase, or TIMP (tissue inhibitors of metalloproteinases), especially TIMP-1, TIMP-2 or TIMP-3.

The active compound (protein B) can also be a protein for relieving damage to the nervous system, for example a growth factor, such as FGF, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), neurotrophin 4 (NT-4) or ciliary neurotrophic factor (CNTF), or a cytokine, or a cytokine inhibitor, which is able to inhibit or neutralize the neurotoxic effect of TNF α , for example TGF β , soluble TNF receptors, IL-10, since IL-10 inhibits the formation of IFN γ , TNF α , IL-2 and IL-4, soluble IL1 receptors, such as IL-1 receptor I or IL-1 receptor II, since soluble IL-1 receptors neutralize the activity of IL-1, IL-1 receptor antagonist or soluble IL-6 receptors.

The active compound (protein B) can also be a protein which stimulates angiogenesis, for example VEGF or FGF.

The active compound (protein B) can furthermore be a protein which lowers blood pressure, for example kallikrein or endothelial cell nitric oxide synthase.

The active compound (protein B) can also be a protein for the therapy of chronic infectious diseases, for example a protein which exhibits cytostatic or cytotoxic effects, or an enzyme which cleaves a precursor of an antiviral or cytotoxic substance into the active substance, or a cytotoxin having an antiviral effect or a growth factor having an antiviral effect. Examples are $IFN\alpha$, $IFN\beta$, $IFN\gamma$, $TNF\beta$, $TNF\alpha$, IL-1 or $TGF\beta$.

The present invention furthermore relates to a nucleic acid construct in which two identical or two different DNA sequences, which encode identical or different active compounds (protein B) [component b) and b'')] are combined.

In order to ensure that both DNA sequences are expressed, the cDNA of an internal ribosome entry site (IRES) is preferably intercalated, as a regulatory element, between the two structures. An internal ribosome entry site makes it possible to express two DNA sequences which are linked to each other by way of an IRES. IRESS of this nature have been described, for example, by Montford and Smith TIG 11, 179 (1995); Kaufman et al., Nucl. Acids Res. 19, 4485 (1991); Morgan et al., Nucl. Acids Res. 20, 1293 (1992); Dirks et al., Gene 128, 247 (1993); Pelletier and Sonenberg, Nature 334, 320 (1988) and Sugitomo et al., BioTechn. 12, 694 (1994). Thus, for example, the cDNA for the polio virus IRES sequence (positions ≤ 140 to ≥ 630 of the 5' UTR [Pelletier and Sonenberg, Nature 334, 320 (1988)] can be used to link the DNA of component c) to the DNA of component d).

Nucleic acid sequences [component c)] which encode the protease-cleavable part structure C:

According to the present invention, part structure C comprises an amino acid sequence which is cleaved by proteases which are formed in tumors or by tumor cells or

inflammatory cells. The nucleic acid sequence for this part structure C is inserted, for example, into the nucleic acid sequence of the naturally occurring precursor (protein BSD, where S is the naturally occurring cleavage sequence) of the relevant active compound (protein B) in place of the cleavage sequence S such that this recombinant nucleic acid expresses protein BCD or B'BCD.

The nucleic acid sequence encoding part structure C is chosen depending on the protease which is predominantly secreted in the tumor or in the inflammation.

The following part structures C may, for example, be employed for the following enzymes [Barrett et al., Mammalian Proteases, Academic Press, London (1980), Panchal et al., Nature Biotechnol. 14, 852 (1996); Pigott et al., Ayad et al., The extracellular Matrix, Academic press (1994); Yoshida et al., Int. J. Cancer 63, 863 (1995), Petersen et al., J. Biol. Chem. 265, 6104 (1990); Cramer et al., J. Urology 156, 526 (1995); Forsgen et al., FEBS Lett. 213, 254 (1987) Zhang et al. Chin. Chem. 41, 1567, (1995)]:

Enzyme							Part structure C cleavage	
	A6	A5	A4	A3	A2	A1	A-1	(A-2)
Plasminogen activator			Cys	Pro Gln Gly Arg Gly	Gly Gly Gly Phe Lys	Arg Arg Arg Lys Arg	Val (Ile)	(Val)
Prostate-specific antigen	Ser Gly Gly	Phe Ser Ser Ile	Pro Arg Ser Gln Ser	Arg Arg Arg Ile Gln Ser	Phe Pro Phe Gln Gln	Lys Tyr Phe Tyr Tyr Leu Tyr	Ile Leu (Ile) Ile Ile Ile Ile	(Ile) (Val) (His) (Val) Val Val Val Val

Enzyme							Part structure C cleavage	
	A6	A5	A4	A3	A2	A1	A-1	(A-2)
Cathepsins			Pro	Arg Lys Lys Ile Arg Gln Lys	Phe Ser Met Arg Ala Ala Leu Lys	Lys Tyr Arg Arg Arg Arg Phe Arg Lys Arg	Ile Met (Ile) Arg (Ile) Arg (Ile) Leu (Ile) Phe (Ile) Leu (Ile) Val (Ile)	Ile (Val)
Stromelysins			Gly Gln Ala Val Leu	Gly Leu Ala Ala Ala	Gly Gly Ala Val Ala	Ala Val Ser Ser Asn	Gln Met Leu Leu	(Leu) (Gln) (Lys) (Lys) (Arg)

Enzyme							Part structure C cleavage	
	A6	A5	A4	A3	A2	A1	A-1	(A-2)
Collagenase I II III VIII XI			Gly	Pro	Gln	Gly	Ile	(Ala)
			Gly	Pro	Gln	Gly	Leu	(Leu)
			Gly	Pro	Gln	Gly	Leu	(Ala)
			Gly	Ile	Ala	Gly	Ile	(Thr)
			Gly	Leu	Pro	Gly	Ile	(Gly)
			Gly	Phe	Pro	Gly	Ile	(Gly)
Plasminogen			Gly	Pro	Ala	Gly	Ile	(Ser)
			Ser	Gly	Thr	Glu	Ile	(Ala)

The amino acid positions (A1-A6 and A-1 and A-2) were defined in accordance with Schechter and Berger, Biochem. Biophys. Res. Comm. 27, 157 (1967).

Nucleic acid sequences [component d]] which encode part structure D:

5 According to the present invention, the nucleic acid sequence [component d]] encodes a peptide (part structure D) which binds to the active compound (part structure B) by way of the part structure C and inactivates this active compound by means of this binding.

10 Preferably, those nucleic acid sequences are used for part structure D which encode part structure D in the naturally occurring precursors (protein BSD), with part structure S being the natural cleavage sequence in protein BSD.

15 The structures of the naturally occurring precursors of active compounds (protein B) have already been reviewed, for example by Bartett et al., Mammalian Proteases, Academic Press, London (1980) in the case of coagulation factors, complement factors and kallikrein, by Callard et al., The Cytokine Facts Book, Academic Press (1994) in the case of interleukins, chemokines and growth factors, and by Denhardt et al., Pharmac. Ther. 59, 329 (1993) in the case of tissue inhibitors of metalloproteinases (TIMPs).

25 When selecting active compounds which do not have any naturally occurring precursors, and in the case of xenogeneic active compounds, use should be made of nucleic acid sequences, as component d), which encode any peptide, preferably, however, of nucleic acid sequences which encode those part structures D which naturally occur in the precursors of human active compounds.

30 In order to facilitate secretion of the protein BCD, or B'BCD, which is expressed by the novel nucleic acid sequence, the homologous signal sequence which may be present in the DNA sequence of component b) can be replaced with a heterologous signal sequence which improves extracellular secretion. Thus, for example, the
35 signal sequence for immunoglobulin [DNA positions ≤ 63 to

5 ≥ 107 ; Riechmann et al., Nature 332, 323 (1988)] or the
signal sequence for CEA [DNA positions ≤ 33 to ≥ 134 ,
Schrewe et al., Mol. Cell Biol. 10, 2738 (1990); Berling
et al., Cancer Res. 50, 5634 (1990)] or the signal
sequence of human respiratory syncytial virus
glycoproteins [cDNA of amino acids ≤ 38 to ≥ 50 or 48 to
65; Lichtenstein et al., J. Gen. Virol. 77, 109 (1996)]
can be inserted.

10 In addition, in order to augment translation, the
nucleotide sequence GCCACC or GCCGCC [Kozak, J. Cell
Biol. 108, 299 (1989)] can be inserted at the 3' end of
the promoter sequence and directly at the 5' end of the
start signal (ATG) of the signal sequence.

Preparation of the novel nucleic acid constructs

15 The novel nucleic acid constructs which have been
described are prepared by linking the individual
components to each other using standard molecular
biological methods.

Applications:

20 The novel nucleic acid construct is particularly well
suited for treating diseases which are accompanied by an
increased local formation of proteases, such as tumor
diseases, leukemias, allergies, autoimmune diseases,
infections, inflammations, transplant rejection
25 reactions, thromboses and blood vessel occlusions and
other disturbances of blood clotting and of blood
circulation, and tissue injuries, including injuries to
the central nervous system and damage to the nervous
system. Administration is effected locally (e.g. onto
30 the skin), nasally, orally, gastrointestinally,
intrabronchially, intravesically, intravaginally, into
the uterus, sub-cutaneously, intramuscularly,
periarticularly, intraarticularly, into the cerebrospinal
fluid, into the brain tissue, into the spinal medulla,

into wounds, intraperitoneally or intrapleurally, or systemically, e.g. intravenously, intraarterially, intraportally or into the heart.

5 In general, the administered composition comprises, where appropriate in addition to the customary additives and auxiliary substances, either the novel nucleic acid construct or a cell which is able to express the novel nucleic acid construct. The administered composition can be administered for the prophylaxis or therapy of a
10 disease, as already described in detail above.

For administration purposes, an effective amount is determined by the skilled artisan considering variables well known in the art such as the nature of the applicable disease or condition, the nature of the
15 patient, mammal or cells being treated and the method of administration.

Moreover, in addition to the methods of administration discussed above, the present invention contemplates the administration of the novel nucleic acid construct to a mammal by ex vivo gene transfer of the
20 cells of the subject mammal in a clinical setting. Such techniques are well known to those of skill in the art. In addition, the present invention contemplates introduction of the novel nucleic acid construct into cells in vivo
25 [Rosenberg et al., *Science* 242:1575-1578 (1988) and Wolff et al., *PNAS* 86:9011-9014 (1989)]. In this regard, the routes of delivery include systemic administration and administration *in situ*. Well-known techniques include systemic administration with cationic liposomes, and
30 administration *in situ* with viral vectors. Any one of the gene delivery methodologies described in the existing art is suitable for the introduction of novel nucleic acid construct into a target cell.

Said cell is prepared, for example, by transforming
35 or transfecting cells with the novel nucleic acid construct using methods known to the skilled person.

Examples of suitable cells are endothelial cells, lymphocytes, macrophages, glia cells, fibroblasts, liver cells, kidney cells, muscle cells, cells of the bone or cartilage tissue, synovial cells, peritoneal cells, skin cells, epithelial cells, leukemia cells and/or tumor cells.

The novel cells are also suitable for preparing the protein which is encoded by the novel nucleic acid construct and which can be used directly as a drug.

The present invention furthermore relates, therefore, to the use of the novel nucleic acid construct for preparing a recombinantly altered cell, with the nucleic acid construct being introduced into the cells, to the use of the novel nucleic acid construct for preparing a protein which is encoded by the nucleic acid construct, with the nucleic acid construct being caused to express in a suitable cell and the protein which is formed being isolated, and to a cell which harbors the novel nucleic acid construct. The above-described cells are the preferred cells.

The following selection can, for example, be made from the above-mentioned examples of promoter sequences and structural genes (for the protein BCD or B'BCD) depending on the nature and site of the disease and on the target cell to be transduced:

Therapy of tumors:

Promoters [component a)]: endothelial cell-specific and cell cycle-specific or cell-nonspecific or muscle cell-specific and cell cycle-specific or tumor cell-specific (solid tumors, leukemias)

Ligands for the following target cells [component b')]: proliferating endothelial cells or stroma cells and muscle cells adjacent to the endothelial cell or tumor cells or leukemia cells.

Structural genes [component b)c)d]]: for coagulation-inducing factors, for complement factors, for angiogenesis inhibitors, for cytostatic and cytotoxic proteins, for inducers of inflammations or for enzymes for activating precursors of cytostatic agents, for example for enzymes which cleave inactive precursor substances (prodrugs) thereby forming active cytostatic agents (drugs).

Therapy of autoimmune diseases and inflammations:

Promoters [component a]]: endothelial cell-specific and cell cycle-specific, or macrophage-specific and/or lymphocyte-specific and/or cell cycle-specific or synovial cell-specific and/or cell cycle-specific.

Ligands for the following target cells [component b')]: proliferating endothelial cells, macrophages and/or lymphocytes or synovial cells.

Structural genes [component b)c)d]]: for the therapy of antibody-mediated autoimmune diseases, for inhibitors of cell proliferation, cytostatic or cytotoxic proteins, enzymes for activating precursors of cytostatic agents or for the therapy of arthritis.

Therapy of damage to the nervous system:

Promoters [component a]]: glia cell-specific, endothelial cell-specific and cell cycle-specific or nonspecific and cell cycle-specific.

Ligands for the following target cells [component b')]: glia cells or proliferating endothelial cells

Structural genes [component b)c)d]]: for neuronal growth factors, for example for cytokines and cytokine inhibitors which inhibit or neutralize the neurotoxic effect of $TNF\alpha$.

Therapy of disturbances of the blood coagulation system and the blood circulation system:

Promoters [component a]]: cell-nonspecific, cell-nonspecific and cell cycle-specific or specific for endothelial cells, smooth muscle cells or macrophages, or specific for endothelial cells, smooth muscle cells or macrophages and cell cycle-specific.

Ligands for the following target cells [component b')]: endothelial cells, proliferating endothelial cells or somatic cells in the vicinity of endothelial cells and smooth muscle cells or macrophages.

Structural genes [component b)c)d]]: for the inhibition of coagulation or for the promotion of fibrinolysis, for angiogenesis factors, for hypotensive peptides, for an antiproliferative, cytostatic or cytotoxic protein or for an enzyme for cleaving precursors of cytostatic agents, thereby forming cytostatic agents, for inhibition of the proliferation of smooth muscle cells following injury to the endothelial layer or for blood plasma proteins, such as C1 inactivator, serum cholinesterase or α 1-antitrypsin.

Therapy of chronic infectious diseases:

Promoters [component a]]: virus-specific, cell-specific or virus-specific or cell-specific and cell cycle-specific.

Ligands for the following target cells [component b')]: liver cells, lymphocyte and/or macrophage, epithelial cell or endothelial cell.

Structural genes [components b)c)d]]: for a protein which exhibits cytostatic or cytotoxic effects, an enzyme which cleaves a precursor of an antiviral or cytotoxic substance thereby forming the active substance, or for antiviral proteins such as antivirally active cytokines and growth factors.

The invention is explained in more detail with the aid of the following examples and figures without restricting it thereto:

Examples

- 5 1. Preparation of a nucleic acid construct encoding prostate-specific antigen (PSA)-activatable FX

 This deals with the preparation of a therapeutic agent for treating prostate carcinoma metastases. Despite the surgical removal of a prostate which has become carcinomatous, metastases of the prostate carcinoma frequently arise which are currently still largely untreatable and which lead to the death of the patient. Such prostate carcinoma metastases induce angiogenesis. Furthermore, prostate carcinoma metastases secrete a tissue-specific enzyme, i.e. prostate-specific antigen (PSA). In accordance with the invention, a nucleic acid construct is prepared which, having been introduced into proliferating endothelial cells, leads to a modified FX coagulation factor being expressed. The modification comprises replacing, in the gene for the natural FX, the nucleotide sequence for the natural cleavage site, whose cleavage results in coagulation-active FXa, with a nucleotide sequence encoding a PSA-specific cleavage site. As a result, the PSA which is secreted by prostate carcinoma metastases is able to specifically activate the modified FX which is secreted by proliferating endothelial cells in the vicinity of the metastases and thereby to initiate the coagulation which leads to the blood supply to the metastasis being interrupted and consequently to necrosis of the metastasis.

 The nucleic acid construct for the PSA-activatable FX is prepared in accordance with a scheme which is depicted in Figure 3.

The DNA sequences of the individual components are joined together, in the 5' to 3' direction, as follows:

Component a), which contains the promoter sequence of the cdc25C gene [nucleic acids: -290 to +121; Lucibello et al., EMBO J. 14, 132 (1995); Zwicker et al., Nucl. Acids Res. 23, 3822 (1995); EMBO J. 14, 4514 (1995)], the sequence GCCACC (Kozak, J. Cell Biol. 108, 229 (1989)) and the cDNA for the immunoglobulin signal peptide [nucleotide sequence ≤ 63 to ≥ 107 ; Riechmann et al., Nature 332, 323 (1988)], is fused to component b)c)d), which contains the cDNA for human FX (nucleotide sequence 1 to ≥ 1468) [Messier et al., Gene 99, 291 (1991)] in which amino acid 194 has been mutated from Arg to Tyr.

The individual components of the construct are linked by way of suitable restriction sites which are introduced at the termini of the different elements by way of PCR amplification. The linking is effected using enzymes which are specific for the restriction sites and which are known to the skilled person, and DNA ligases. These enzymes can be obtained commercially.

The nucleotide construct which has been prepared in this way is cloned into pUC 18/19 or Bluescript-derived plasmid vectors.

2. Expression in human embryonic kidney cells

Proliferating human embryonic kidney cells [HEK 293; Racchi et al., J. Biol. Chem. 268, 5735 (1993)] which are being maintained in culture are transfected with the above-described plasmid using the method known to the skilled person [Graham and van der Eb, Virol. 52, 456 (1973)].

The mutated factor X is purified from the supernatant from approx. 10^7 transfected HEK 293 cells [Watzke et al., J. Clin. Invest. 88, 1685 (1991)] and assayed in a coagulation test for factor X with and without the

addition of PSA. Purified PSA is obtained from Chemicon (Temecula, CA, USA).

In this test, the coagulation defect in human FX-deficient plasma is counterbalanced by functionally active FXa.

Nonmutated (wild-type) FX (which is activated by Russel's viper venom) is employed as a positive control. In addition to the test mixture lacking PSA, a mock preparation from the supernatant from untransfected HEK 293 cells is used as a negative control.

The coagulation activity of the mutated FX is measured by recalcification time (Seitz R et al., Int. J. Cancer 53:514-520, 1993). 100 μ l of FX-deficient plasma (Behringwerke, Marburg) are incubated, at 37°C for 120 sec, with 100 μ l of the FX preparation from the cell supernatant. The FX preparation contains PSA as activator. No PSA is added in the case of the negative control. FX (wild-type) and Russel's viper venom (RVV) are employed as the positive control. The coagulation reaction is augmented by adding 100 μ l of 0.02 M CaCl₂ and determined in a coagulometer.

The following results are obtained:

The negative controls without any activation of coagulation give a coagulation time of approx. 200 sec. By contrast, significantly shorter coagulation times, of 50 sec, are achieved when activated FX (mutated FX and PSA or wild-type FX and RVV) is used.

It can be concluded from this that the transduced HEK 293 cells express mutated FX which, in the added presence of PSA, counterbalances the coagulation defect of FX-deficient plasma.

3. Expression in human endothelial cells

Human umbilical cord endothelial cells which are being maintained in culture are transfected with the

above-described plasmid using the method known to the skilled person (Lucibello et al., EMBO J. 14, 132 (1995)).

5 In order to check cell cycle specificity, endothelial cells are synchronized in G0/G1 by withdrawing methionine over a period of 48 hours. After staining with Hoechst 33258 (Hoechst AG, Frankfurt), the DNA content of the cells is determined in a fluorescent-activated cell sorter (Lucibello et al., EMBO J. 14, 132 (1995)).

10 The expression of the nucleic acid construct is assayed in the supernatant from the endothelial cells in analogy with the investigation carried out on the HEK 293 cells.

The following results are obtained:

15 The protein which is expressed by the transfected endothelial cells counterbalances the coagulation defect of FX-deficient plasma, in contrast to mock preparations from the supernatant from untransfected endothelial cells.

20 A markedly higher concentration of mutated FX can be detected in the supernatant from proliferating, transduced endothelial cells (DNA > 2S) as compared with the supernatant from endothelial cells which have been synchronized in G0/G1 (DNA = 2S).

25 Consequently, the above-described nucleic acid construct leads to the gene for the mutated FX being expressed in a cell cycle-dependent manner in endothelial cells, and this mutated FX can be activated by PSA such that it brings about coagulation in FX-deficient plasma.

30 Federal Republic of Germany priority application, DE 19701141.1, filed January 16, 1997, including the specification, drawings, claims and abstract, is hereby incorporated by reference.

What Is Claimed Is:

1. A nucleic acid construct for expressing an active substance which is activated by an enzyme which is released from mammalian cells, wherein said construct comprises the following nucleic acid sequences in the following order:

a) at least one promoter element operably linked to;

b) at least one nucleic acid sequence which encodes an active compound, wherein said active compound is endogenous to mammals, operably linked to;

c) at least one nucleic acid sequence which encodes an amino acid sequence cleavable specifically by an enzyme which is released from a mammalian cell, operably linked to;

d) at least one DNA sequence which encodes a polypeptide which is bound to said active compound by said cleavable amino acid sequence and inhibits the activity of said active compound, and wherein said nucleic acid component c) does not naturally occur as operably linking said nucleic acid sequence b) to said nucleic acid d).

2. A nucleic acid construct as claimed in claim 1, wherein said enzyme is a protease.

3. A nucleic acid construct as claimed in claim 1, wherein said enzyme is a prostate specific antigen, a plasminogen activator, a cathepsin or a matrix metalloproteinase.

4. A nucleic acid construct as claimed in claim 1, wherein said mammalian cells are tumor cells, leukemia cells, endothelial cells, macrophages, lymphocytes,

muscle cells, epithelial cells, glia cells, synovial cells or virus-infected cells.

5. A nucleic acid construct as claimed in claim 1, wherein said nucleic acid construct further comprises a nucleic acid sequence operably linked to said construct of claim 1, wherein said nucleic acid sequence encodes a ligand which binds said active compound to a target structure.

6. A nucleic acid construct as claimed in claim 1, wherein said nucleic acid sequences b) and d) of claim 1 encode parts of a natural precursor of a protein active compound, wherein the nucleic acid sequence encoding the cleavage sequence naturally occurring between said nucleic acid sequences b) and d) has been replaced by said nucleic acid sequence c), which does not naturally occur between said nucleic acid sequences b) and d).

7. A nucleic acid construct as claimed in claim 1, wherein said polypeptide encoded by said nucleic acid sequence d) is part of a natural precursor of a protein active compound.

8. A nucleic acid construct as claimed in claim 1, wherein said construct is operably inserted into a plasmid or a viral vector.

9. A nucleic acid construct as claimed in claim 1, wherein said nucleic acid sequence a) is a promoter sequence which can be activated nonspecifically, cell-specifically, virus-specifically, metabolically, cell cycle-specifically or by tetracycline.

10. A nucleic acid construct as claimed in claim 1, wherein said nucleic acid sequence a) comprises at least two identical or two different promoter sequences.

11. A nucleic acid construct as claimed claim 9, wherein said nucleic acid sequence a) is activated in endothelial cells, in cells adjoining activated endothelial cells, in muscle cells, in leukemia cells, in tumor cells, in glia cells, in lymphocytes, in macrophages or in synovial cells.

12. A nucleic acid construct as claimed in claim 1, wherein said active compound activates or inhibits a biological activation cascade or is an active component of this cascade, or activates or inhibits the coagulation system, activates fibrinolysis, activates the complement system or activates the kinin system, or is an enzyme which converts the inactive precursor of a pharmacological substance into the active substance, or which itself is a pharmacologically active substance.

13. A nucleic acid construct as claimed in claim 12, wherein said active compound is a coagulation factor which is selected from the group consisting of thrombin, factor Va, factor VIIa, factor IXa, factor Xa, TF coagulation-active fragments or factor XIIa; thrombin which is mutated in the region of the Arg-Thr cleavage site (amino acid position 327/328); a fibrinolytic protein which is selected from urokinase, tPA or functional hybrids thereof; a complement factor which is selected from C5b, C3b or functional cleavage products thereof; an antithrombotic protein which is selected from protein C, C-1S inhibitor, α 1-antitrypsin, hirudin, AT-III, TFPI, PAI-1, PAI-2 or PAI-3; a kallikrein; a cytostatic, cytotoxic or inflammation-eliciting protein; an antiangiogenic protein; an immunomodulatory protein;

an antiinflammatory protein; a protein which relieves damage to the nervous system; a protein which inhibits or neutralizes the neurotoxic effect of TNF α ; an angiogenesis-stimulating protein; a hypotensive protein; an antiviral protein; a cytokine; an interferon; a tumor necrosis factor; oncostatin M or LIF; a cytokine receptor; the moiety of a cytokine receptor which is external to the cell; a cytokine antagonist; a growth factor; a growth factor receptor; the moiety of a growth factor receptor which is external to the cell; a chemokine; angiostatin; platelet factor 4; TIMP-1, TIMP-2 or TIMP-3; a nitroreductase; a β -glucuronidase; a carboxypeptidase; a β -lactamase; a cytosine deaminase; a catalase; a peroxidase; a phosphatase; an oxidase; kallikrein or an endothelial cell nitric oxide synthase.

14. A nucleic acid construct as claimed in claim 1, which further comprises a nucleic acid sequence b') which encodes a ligand which binds to a cell membrane receptor, a cell membrane antigen, a cell membrane-located adhesion molecule, or to the extracellular matrix or component thereof.

15. A nucleic acid construct as claimed in claim 14, wherein said ligand is an antibody or an antibody fragment which binds specifically to a cell membrane antigen or to an antigen on the extracellular matrix, or is a polypeptide which binds to receptor on the cell membrane wherein said polypeptide is a growth factor, a cytokine, an interferon, a tumor necrosis factor, a chemokine, a receptor-binding part sequence of these ligands, a peptide hormone, angiotensin, kinin, folic acid, an adhesion molecule or the part sequence of the adhesion molecule which binds to the corresponding adhesion molecule or to the extracellular matrix, an extracellular moiety of an Fc receptor, a glycoprotein of

a virus, a part sequence of the glycoprotein which binds to these cells, the transmembrane domain of a receptor or of a viral glycoprotein, or a glycopospholipid anchor.

16. A nucleic acid construct as claimed in claim 14, wherein said ligand binds to activated or proliferating endothelial cells, to tumor cells, to muscle cells, preferably smooth muscle cells, to fibroblasts, to macrophages, to lymphocytes, to liver cells, to kidney cells, to synovial cells, to inflammatory cells, to virus-infected cells, to bronchial epithelial cells, to glia cells or to leukemia cells.

17. A nucleic acid construct as claimed in claim 14, wherein the construct comprises at least two identical or different nucleic acid sequences b)c)d) or b')b)c)d), which nucleic acid sequences are linked to each other by way of an internal ribosomal entry site.

18. A process for preparing a nucleic acid construct according to claim 1, which comprises operably linking said nucleic acid sequences of claim 1.

19. A method for the treatment or prophylaxis of tumors, leukemias, allergies, autoimmune diseases, infections, inflammations, transplant rejection reactions, thromboses, blood vessel occlusions, blood coagulation, blood circulation disturbances, injuries to tissues, or damage to the nervous system, comprising administering to a mammal an effective amount of a polypeptide expressed by the nucleic acid construct of claim 1.

20. A method for preparing a recombinantly altered cell, comprising transducing a suitable cell with said nucleic acid construct of claim 1.

21. A method for preparing a polypeptide which is encoded by said nucleic acid construct of claim 1, comprising transducing a suitable cell with said construct, expressing said polypeptide in said cell, and isolating said expressed polypeptide.

22. The method of claim 20, wherein said cell is an endothelial cell, a lymphocyte, a macrophage, a glia cell, a fibroblast, a liver cell, a kidney cell, a muscle cell, a cell of the bone or cartilage tissue, a synovial cell, a peritoneal cell, a skin cell, an epithelial cell, a leukemia cell or a tumor cell.

23. The method of claim 21, wherein said cell is an endothelial cell, a lymphocyte, a macrophage, a glia cell, a fibroblast, a liver cell, a kidney cell, a muscle cell, a cell of the bone or cartilage tissue, a synovial cell, a peritoneal cell, a skin cell, an epithelial cell, a leukemia cell or a tumor cell.

24. A cell transduced with said nucleic acid construct of claim 1.

25. A protein encoded by said nucleic acid construct of claim 1.

Abstract of the Disclosure

The invention relates to a nucleic acid construct for expressing an active substance which is activated by an enzyme which is released from mammalian cells, which construct comprises the following components: a) at least one promoter element, b) at least one DNA sequence which encodes an active compound (protein B), c) a least one DNA sequence which encodes an amino acid sequence (part structure C) which can be cleaved specifically by an enzyme which is released from a mammalian cell, and d) at least one DNA sequence which encodes a peptide or protein (part structure D) which is bound to the active compound (protein B) by way of the cleavable amino acid sequence (part structure C) and inhibits the activity of the active compound (protein B), and also to the use of the nucleic acid construct for preparing a drug for treating diseases.

Figure 1

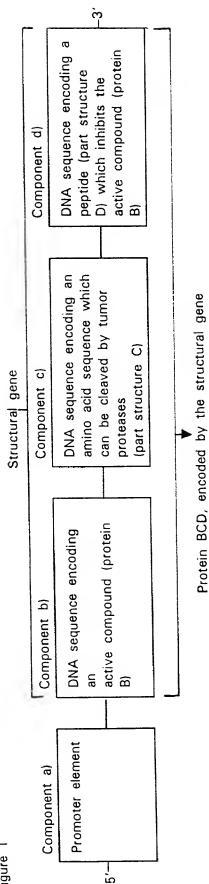


Figure 2

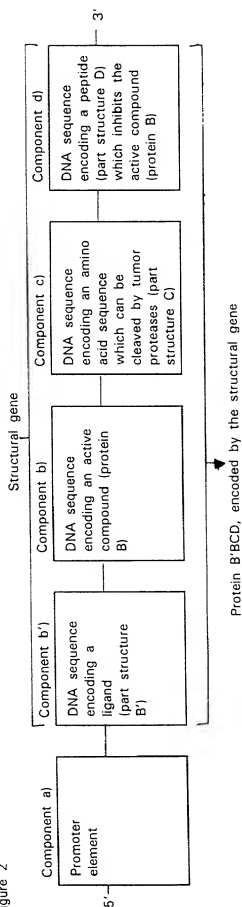
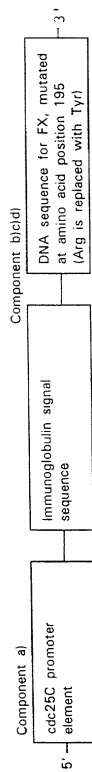


Figure 3



DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NUCLEIC ACID CONSTRUCT FOR EXPRESSING ACTIVE SUBSTANCES WHICH CAN BE ACTIVATED BY PROTEASES, AND PREPARATION AND USE

the specification of which is attached hereto unless the following box is checked:

☒ was filed on January 16, 1998 as United States Application Number or PCT International Application Number 09/008,308 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
<i>197 01 141.1</i>	<i>Federal Republic of Germany</i>	<i>16 January 1997</i>	<i>YES</i>

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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